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Different Expression Analysis in Fruit Softening and Ethylene Biosynthetic Pathways in Peaches of Different Flesh Textures

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Abstract

The aim of our study was to assess differences in the expression of genes involved in fruit softening and ethylene biosynthetic pathways under different temperature storage conditions. Different peach cultivars of 'Xiacui' and 'Yumyeong', which are stonyhard, 'Yinhualu', which is soft-melting, 'Hujing Milu', which is hard-melting, and 'Baby Gold 6', which is non-melting at 80% ripening, were collected as test materials. The results showed that only slight ethylene production was detected after harvesting of 'Yumyeong' and 'Xiacui' under either a room temperature (25 °C) or low temperature (4 °C). The fruit firmness of stonyhard cultivars was retained at a high level under room temperature over time, whereas a low temperature induced 'Yumyeong' fruit to soften. Quantitative real-time PCR results indicated that the *PpACS1* gene was highly expressed in soft-melting, hard-melting and non-melting cultivars; however, expression was extremely low in stonyhard peaches. *PpACS2* or *PpACS3*, however, was not detected in all five cultivars. Interestingly, cold treatment significantly decreased firmness along with *endo-PG* expression obviously up-regulated in 'Yumyeong', but not in 'Xiacui' peaches. In conclusion, this study revealed that fruit softening of peaches with different flesh textures was closely related to ethylene biosynthesis during the storage period, which was controlled via regulating relevant gene expression levels under different storage temperatures.

Keywords: *Prunus persica*; ethylene; biosynthetic pathway; soften; gene expression

1. Introduction

Peach flesh textures are divided into four types; i.e., soft-melting, hard-melting, non-melting and cotton-like (Wang et al., 2005). Yoshida, a Japanese peach expert, proposed a new type of peach flesh texture; i.e., stonyhard, in which the fruit flesh does not soften in the developing period and after harvest, but changes color normally and has a good taste (Haji et al., 2001, 2004). Genetic analysis showed that stonyhard (*hd*) is a recessive genetic locus that is different from melting (*M*)/non-melting (*m*) (Yoshida, 1976; Haji et al., 2005). In general, the peach belongs to a climacteric fruit. During ripening, the fruit texture changes, resulting in a decrease in firmness accompanied by an increase of ethylene release and up-regulated expression of genes.

ACC (a direct precursor of ethylene biosynthesis) synthase (ACS) and ACC oxidase (ACO) are key enzymes in the regulation of ethylene biosynthesis (Yang and Hoffman, 1984; Xu et al., 1998; Yin et al., 2009). ACS and ACO are encoded by genes of multiple families and their expression levels are under the combined effects of developmental processes and environmental factors (Kende, 1993; Zarembinski and Theologis, 1994). Kan et al. (2012) found that soft-melting peach 'Yuhua 3' decreased its firmness rapidly after harvesting, significantly increasing ethylene production. Also, the expression levels of *ACS* and *ACO* genes increased first and then decreased. Instead, the firmness of non-melting 'Jianayan' peach fruits changed slowly during maturation and only decreased in late maturation, then maintained overall at a high level, and *ACS* and *ACO*

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expression levels were relatively low. Hayama et al. (2006) showed that the melting 'Akatsuki' fruits softened quickly after harvesting and *endo-PG* gene expression increased rapidly, while the stonyhard type peach 'Manami' showed no significant changes in fruit flesh firmness after harvesting and *endo-PG* gene expression stayed at a low level.

This study used gas chromatography to investigate the ethylene release mechanisms of various peach flesh textures after harvesting. We combined firmness changes and ethylene biosynthesis pathways with analysis of softening-related gene expression to further explore the mechanisms of peach fruit flesh softening. Thus, our results provide a theoretical basis for research on peach fruit flesh softening and ethylene biosynthesis mechanisms, offering a reference for peach preservation.

2. Materials and methods

2.1. Materials and processing

Five varieties of peach fruits were tested. Soft-melting peach 'Yinhualu' softens quickly after harvest and has a very short shelf life; i.e., only 2–3 d, and cannot tolerate storage. Hard-melting peach 'Hujing Milu' has a longer shelf life of 4–5 d. Non-melting peach 'Baby Gold 6' maintains a stable firmness after harvest. Even if it fully matures, its firmness does not decrease significantly. 'Yumyeong', of the stonyhard type, recovers softening ability by exogenous ethylene, a typical stonyhard melting (able to recover) type, while 'Xiacui' belongs to a stonyhard non-melting (not restored) type (Haji et al., 2005). Fruit fleshes of 'Yumyeong' and 'Xiacui' show a high level of firmness and soften slowly after picking, without a significant change in firmness, and the shelf life is up to about 15 d.

The five peach cultivars were grown in the test garden of Jiangsu Academy of Agricultural Sciences. The fruit trees were robust and naturally open center types. There were three experimental trees for each species. The tested plants were cultivated according to conventional management measures.

In 2014, we harvested fruits above the middle of the canopy in good lighting conditions and at 80% ripening. Immediately after the harvest, fruits were brought back to the lab and then pest-free fruits of uniform size and relatively uniform maturity were chosen for later tests.

Tests were performed as below. A single layer of fruits on a plastic tray were placed inside a polyethylene plastic bag with an inner-lining thickness of 0.04 mm. The bag was opened and placed at room temperature (25 ± 1) °C on a shelf (approximately 75% humidity). Another group of single-layer fruits on a tray was placed in a perforated polyethylene plastic bag with the inner-lining thickness of 0.04 mm. This bag was lightly tied and placed in a refrigerator (4 ± 0.5) °C at a relative humidity of 75%. Each group had 70 fruits and experiments were repeated three times, which included a total of 210 fruits. Each time 10 fruits were randomly chosen and the related parameters were measured. The measurements were repeated three times.

2.2. Measurement of ethylene release rate and firmness

Ten fruits were placed in a 5 L sealed container for 2 h and the extracted gas was measured for ethylene content; measurements were repeated three times. The ethylene release rate ($\mu L \cdot kg^{-1} \cdot h^{-1}$) was represented by the released amount of ethylene per unit time per unit fresh weight (FW) of fruits. Ethylene was measured with a GC-7890A gas chromatograph (Agilent) and chromatographic conditions were: FID detector, Hp-Plot q capillary column (20 m × 0.53 mm × 20 μm), split ratio 10, a carrier gas He, 40 °C column temperature, 220 °C detector temperature, and 1 mL injection volume. Fresh fruits were used for measurements and measurements were repeated three times.

A TA.XT. Plus Texture Analyzer was used to measure the firmness of peeled fruits in the middle of both sides of the fruit suture. The probe diameter was 8 mm and the test depth was 5 mm at a 1 $mm \cdot s^{-1}$ penetration rate. The average of two points was taken for the firmness of each peeled fruit.

2.3. Analysis of related gene expression

Total RNA was extracted using a modified CTAB method (Shi and Zhuo, 2006). DNA contamination was removed using DNase I (Promega Corporation) digestion. RNA reverse transcription was performed using a PrimeScript™ Double Strand cDNA Synthesis Kit (TaKaRa). All procedures followed the instructions of the manufacturers.

According to the recorded peach genomic sequences, Primer 5.0 was used to design specific primer sequences for *ACTIN*, *ACS1*, *ACS2*, *ACS3*, *ACO1* and *endo-PG* 6 genes (Table 1). Primers were synthesized by Shanghai Invitrogen Biotechnology. SYBR Green (TaKaRa) was used as a fluorescent dye. Fluorescence quantitative analysis was performed with an ABI 7500 quantitative real-time PCR (qRT-PCR) instrument. The amplification system and reaction procedures were similar to the method of Guo et al. (2013) and were repeated three times. The $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001) was used to analyze the gene expression levels and internal *ACTIN* expression (Tong et al., 2009) was used as a standard to determine the expression levels of target genes.

Table 1 Sequences of the primers used for qRT-PCR

Gene	Forward primer (5′—3′)	Reverse primer (5′—3′)	Fragment length/bp
PpACTIN	GTTATTCTTCATCGGCGTCTTCG	CTTCACCATTCCAGTTCCATTGTC	112
PpACS1	GGCAAGGTTCCTGGAGACAA	CACAATCACACGCCAAAGCA	187
PpACS2	TGCACAGCAGCAGGAGTAAA	CCAGGATCAGCCAAGCAGAA	203
PpACS3	ATGCTGGGTTGTTTTGCTGG	AACCTGGTTCAGAGCAGTGG	147
PpACO1	GCAACTACCCTCCTTGTCCC	TGGCCATCTTTGAGGAGCTG	127
endo-PG	ACAACATTGTGGTGAGTGGA	CCATCGGTGTTAGGGCTGTT	130

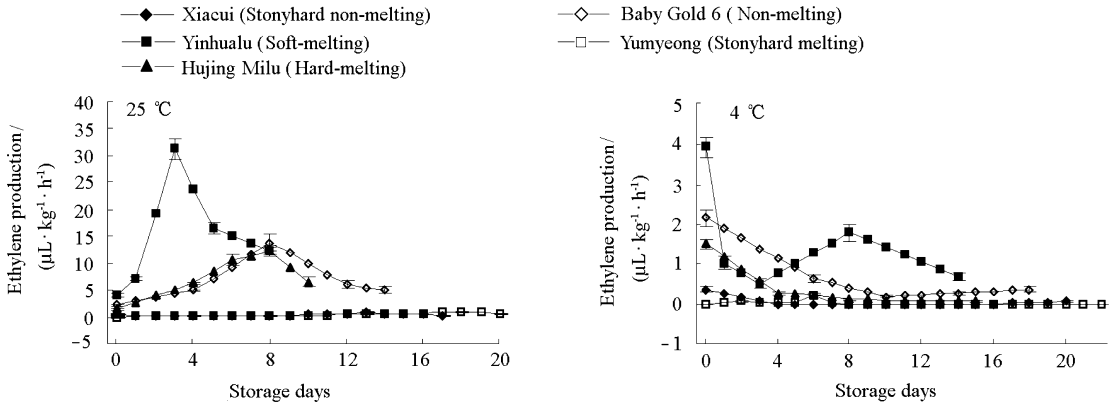

Fig. 1 Changes in ethylene production of different peach cultivars

3. Results

3.1. Changes in ethylene release rates

As shown in Fig. 1, with storage at 25 °C, the ethylene release rate of fruits from soft-melting peach 'Yinhualu', hard-melting peach 'Hujing Milu', and non-melting peach 'Baby Gold 6' increased first and then displayed a falling trend, accompanied by a typical ethylene release peak. 'Yinhualu' peaches rapidly increased ethylene release after harvesting, and reached a peak release of 31.25 $\mu L \cdot kg^{-1} \cdot h^{-1}$ after 3 d of storage. 'Hujing Milu' and 'Baby Gold 6' peaches released a low level of ethylene at the early storage stage after harvesting and accelerated after 4 d of storage. Stonyhard 'Xiacui' and 'Yumyeong' had a very low release rate, without a typical ethylene release peak.

A 4 °C low temperature significantly inhibited the ethylene release rate of different varieties of fruits. During the initial storage at 4 °C, 'Yinhualu', 'Hujing Milu', and 'Baby Gold 6' peach fruits rapidly reduced ethylene production and only 'Yinhualu' showed a peak ethylene release, but only to 1.79 $\mu L \cdot kg^{-1} \cdot h^{-1}$. Stonyhard 'Xiacui' and 'Yumyeong' fruits maintained very low levels of ethylene biosynthesis.

3.2. Changes in fruit firmness

As shown in Fig. 2, under the storage condition of a 25 °C ambient temperature, soft-melting 'Yinhualu' and hard-melting 'Hujing Milu' peach fruits quickly softened during early storage. Non-melting 'Baby Gold 6' softened slowly and maintained relatively stable fruit firmness (2.95–2.39 $kg \cdot cm^{-2}$). Stonyhard 'Xiacui' and 'Yumyeong' decreased firmness slowly and maintained an overall high level of firmness. At the end of storage, 'Yinhualu', 'Hujing Milu', 'Baby Gold 6', 'Xiacui' and 'Yumyeong' fruits demonstrated 78.5%, 91.6%, 19.0%, 19.7% and 24.8% of fruit firmness declining degrees, respectively.

With a 4 °C storage condition, 'Yinhualu' and 'Hujing Milu' peaches decreased their fruit softening rates. 'Hujing Milu' transiently showed the phenomenon of increased fruit firmness at 6–8 d. The fruit firmness of 'Baby Gold 6' at the end of storage was higher than that on day 0. 'Xiacui' changed fruit firmness slightly and remained at a high level. 'Yumyeong' decreased fruit firmness rapidly, from 6.42 $kg \cdot cm^{-2}$ at the initial storage down to 3.11 $kg \cdot cm^{-2}$, which was a decrease of 51.6%.

These results demonstrated that a low temperature alleviates the softening process of soft-melting 'Yinhualu' and hard-melting 'Hujing Milu' peaches, but induced softening of stonyhard 'Yumyeong' fruits.

3.3. Related gene expression analysis

3.3.1. PpACS1

As shown in Fig. 3, under the storage condition of room temperature (25 °C), the *PpACS1* gene transcription level in

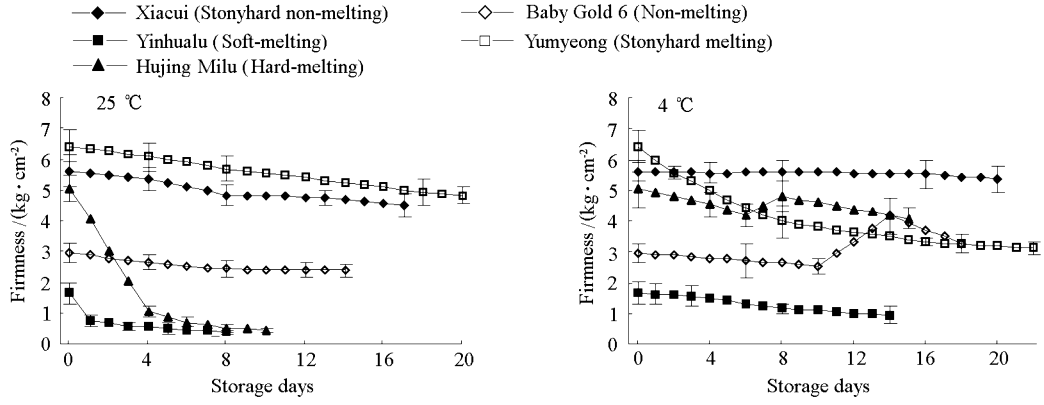

Fig. 2 Changes in flesh firmness of different peach cultivars

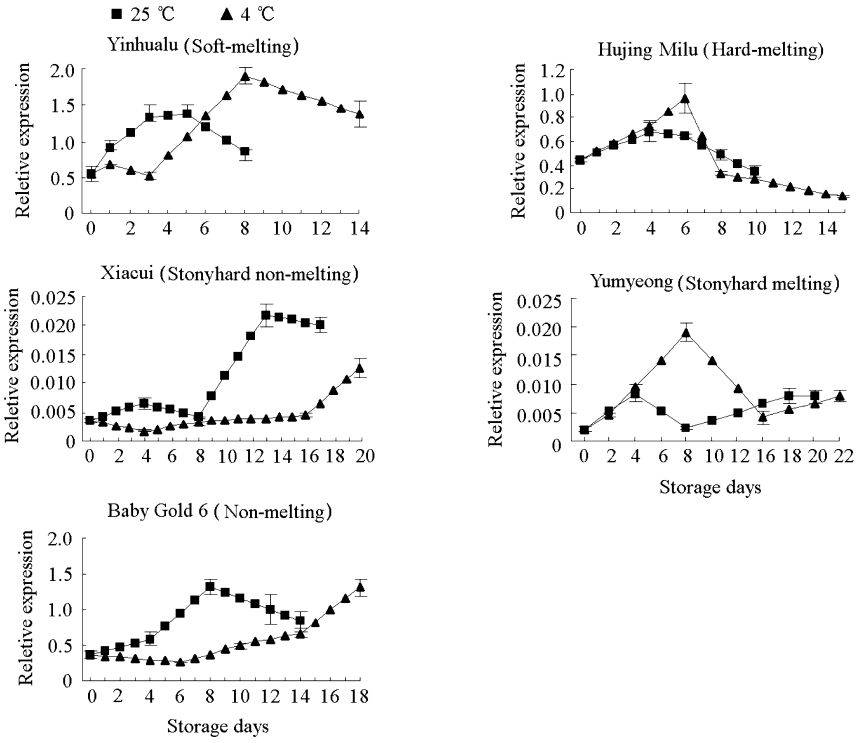

Fig. 3 Expression analysis of *PpACS1* in different peach cultivars

soft-melting 'Yinhualu' peach fruits increased steadily within 0–3 d of storage, reached a peak on 5 d of storage, maintained at a stable level on 3–5 d, and decreased significantly after 5 d. *PpACS1* expression in hard-melting 'Hujing Milu' and non-melting 'Baby Gold 6' increased first and then decreased. Relatively speaking, stonyhard 'Xiacui' and 'Yumyeong' fruits had very low levels of *PpACS1* transcription.

At the storage condition of 4 °C, the *PpACS1* gene expression levels of 'Yinhualu' and 'Baby Gold 6' fruits were substantially increased in the middle and later storage stages. The *PpACS1* level in 'Hujing Milu' rapidly arose, reaching a peak within 0–6 d, and then decreased. The *PpACS1* level in 'Xiacui' and 'Yumyeong' fruits remained low even though there was some fluctuation.

The above data indicated that, under both storage conditions, *PpACS1* gene expression was inhibited in stonyhard peaches compared to that of soft-melting, hard-melting and non-melting peaches.

3.3.2. PpACO1

As shown in Fig. 4, under storage conditions with room temperatures (25 °C), the *PpACO1* transcription level in soft-melting 'Yinhualu' and hard-melting 'Hujing Milu' fruits showed a significant increasing trend. In non-melting 'Baby Gold 6', *PpACO1* transcription gradually increased 8 d after harvest and maintained a stable level after reaching a peak. Stonyhard 'Xiacui' and 'Yumyeong' fruits maintained a lower level of *PpACO1* transcription.

At a 4 °C storage condition, the *PpACO1* transcription levels of 'Yinhualu' and 'Baby Gold 6' fruits were significantly lower than at room temperature storage. The highest value of *PpACO1* relative expression in 'Hujing Milu' was only 46% of that under room temperature storage conditions. The *PpACO1* expression in 'Yumyeong' and 'Xiacui' fruits increased first and then decreased, and their peak expressions were 4.3 times and 4.1 times of those on 0 d of storage, respectively, and 2.0 times and 1.8 times of those under room temperature storage conditions, respectively.

The results showed that under storage conditions of room temperature, the *PpACO1* transcription level in stonyhard peaches was lower than other melting types of peaches. Low temperature suppressed *PpACO1* gene expression in soft-melting, hard-melting and non-melting peach fruits, but induced its expression in stonyhard peaches.

3.3.3. endo-PG

As shown in Fig. 5, under room temperature conditions (25 °C), *endo-PG* gene transcription levels in soft-melting 'Yinhualu' and hard-melting 'Hujing Milu' fruits gradually increased with the extension of storage times, and declined after reaching a peak. Stonyhard 'Xiacui' and 'Yumyeong' showed lower expression levels during the entire storage process and non-melting 'Baby Gold 6' expressed very low levels of *endo-PG*.

After 0–3 d storage at 4 °C, *endo-PG* transcription levels in 'Yinhualu' peaches were low, showed very little change, rapidly rose and peaked on 8 d, and the expression level was 6.3 times of that on 0 d of storage. Hard-melting 'Hujing Milu' and stonyhard non-melting (not restored) 'Xiacui' fruits maintained low levels of *endo-PG* expression. *endo-PG* expression in stonyhard melting (able to recover) 'Yumyeong' fruits rapidly accumulated during 0–8 d of storage and reached a peak, which was 6.4 times of the peak at room temperature.

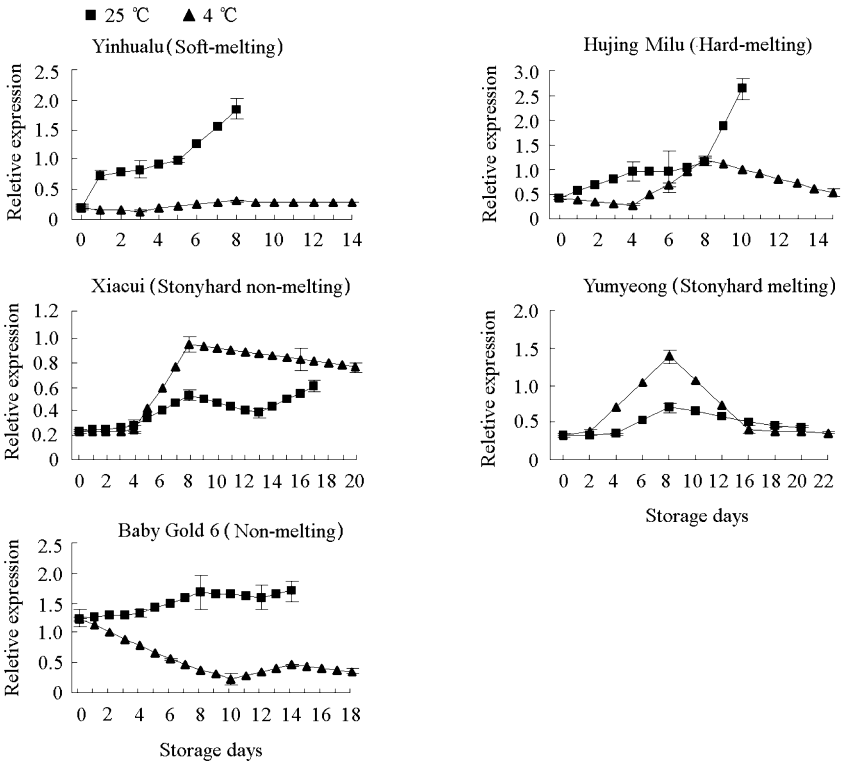

Fig. 4 Expression analysis of *PpACO1* in different peach cultivars

The data indicated that under both storage temperatures, *endo-PG* gene expression levels in non-melting fruits were very low. Low temperatures inhibited the expression of *endo-PG* genes in hard-melting and stonyhard non-melting types of peach fruits, and induced *endo-PG* gene expression in stonyhard melting type peaches.

In addition, this study did not detect the expression of *PpACS2* and *PpACS3* genes in tested fruits.

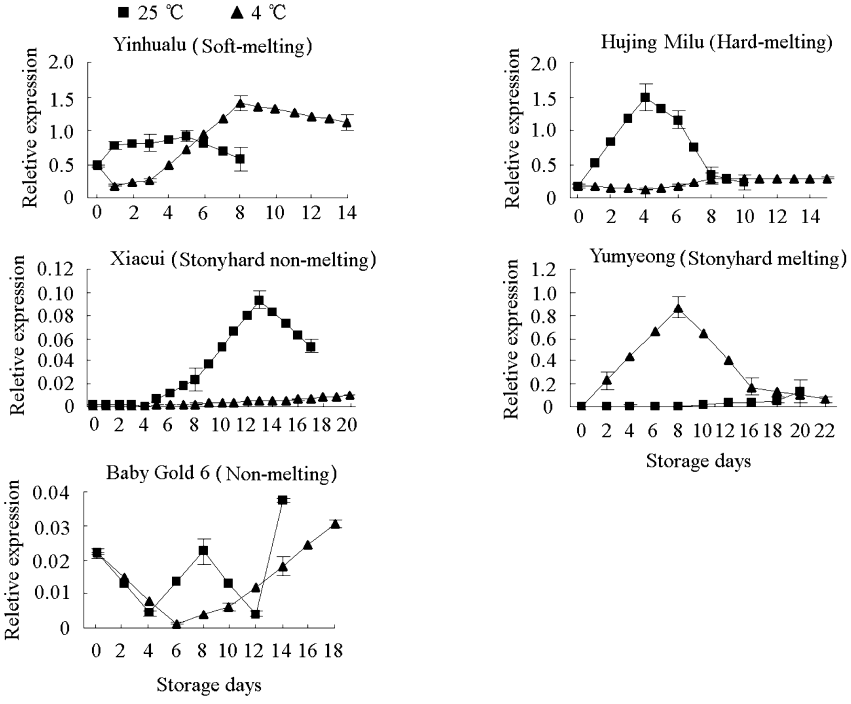

Fig. 5 Expression analysis of *endo-PG* in different peach cultivars

4. Discussion

Peach fruits of the stonyhard type either do not synthesize or synthesize very little ethylene during maturation (Haji et al., 2001). After stonyhard type 'Yumyeong' fruits were treated with exogenous ACC, they recovered the capacity for ethylene synthesis, suggesting that ACC synthase is a key enzyme of stonyhard type peaches in the synthesis of ethylene (Haji et al., 2003). Further research from Tatsuki et al. (2006) showed that inhibition of *PpACS1* expression was the main cause of stonyhard type peaches with an ethylene biosynthesis blockade. This study found that, under a room temperature condition, 'Yinhualu' and 'Hujing Milu' peaches both demonstrated a normal ethylene release peak, and the release trend was consistent with the *PpACS1* gene transcription levels. In addition, the *PpACS1* gene was highly expressed, but was inhibited in stonyhard type peaches and was maintained at very low levels throughout the storage process, which was consistent with previous studies (Tatsuki et al., 2006). However, compared to the non-melting peach 'Jianayan' (Kan et al., 2012), 'Baby Gold 6' peaches had a high ethylene release peak that reached 13.73 $\mu L \cdot kg^{-1} \cdot h^{-1}$, and its relative expressions of *PpACS1* and *PpACO1* were also higher, which may be due to species differences. At low temperatures, soft-melting, hard-melting and non-melting peach fruits produced significantly less ethylene than at room temperature, which was consistent with previous reports (Mao and Zhang, 1999; Ma et al., 2003; Gao et al., 2009; Guo et al., 2009). Although *PpACS1* gene expression showed a higher peak in the middle and later storage stages, ethylene release remained at very low levels, indicating that ethylene biosynthesis was regulated by multiple genes and varied because of the effect of the external storage environment. Stonyhard type 'Xiacui' and 'Yumyeong' fruits did not show a significant increase in *PpACS1* gene expression levels during the entire low temperature storage stage, and *PpACS1* expression was maintained at a very low level.

Under both storage temperatures, *PpACS2* and *PpACS3* gene expressions were not detected in 5 peach fruit varieties during the entire time of storage, which was consistent with the research results of Tatsuki et al. (2006) for melting 'Akatsuki' and stonyhard 'Yumyeong', 'Odoroki' and 'Manami' peaches, indicating *PpACS2* and *PpACS3* genes were not expressed or were expressed at very low levels in ripened peach fruits. The *PpACO1* gene also had an important influence on the biosynthesis of ethylene in the ripening process of peach fruits (Callahan et al., 1992; Lester et al., 1994; Tonutti et al., 1997; Mathooko et al., 2001). This study found that low temperatures inhibited the expression of the *PpACO1* gene in soft-melting, hard-melting and non-melting peach fruits, and induced its expression in stonyhard peaches. This may be due to different response mechanisms between stonyhard and non-stonyhard type peach fruits in response to low temperature stress. This question awaits further study.

Fruit softening is an extremely complex process involving cell wall degradation, co-regulated by cell wall modifying enzymes (Brownleader et al., 1999; Brummel and Harpster, 2001). Also, increased endo-PG activity was considered obligate for peach fruit softening and pulp dissolution (Fishman et al., 1993). This study found that low temperature delayed the softening process of soft-melting 'Yinhualu' fruits and significant softening in fruits was shown until 3 d of storage, which may be caused by the inhibition of *endo-PG* gene expression at the early of storage. The hard-melting peach 'Hujing Milu' did not significantly reduce fruit firmness at the storage condition of a low temperature, which was in stark contrast with its performance under a room temperature condition, and the relative expression of the *endo-PG* gene was maintained at a very low level compared to a room temperature condition. The *endo-PG* relative expression level in the non-melting peach 'Baby Gold 6' was at very low levels under two storage conditions. At the same time, its fruit flesh did not show obvious tissue softening. With later storage at a low temperature, a significant increase in fruit firmness of 'Baby Gold 6' fruits may be due to chilling injury. Interestingly, fruit firmness of stonyhard melting type 'Yumyeong' decreased slowly under room temperature conditions, but the fruit softening rate significantly accelerated because of induction under cold temperatures, which may be related to an upregulated *endo-PG* gene expression level stimulated by a low temperature. However, the same situation did not occur in stonyhard non-melting type 'Xiacui' and the fruit firmness was maintained at a high level in both storage conditions, which may be due to inhibited *endo-PG* gene expression in 'Xiacui' fruits. Although both are stonyhard fruit types, there may be differences in the softening mechanism, which await further study.

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References

Brownleader, M.D., Jackson, P., Mobasheri, A., Pantelides, A.T., Sumar, S., Trevan, M., Dey, P.M., 1999. Molecular aspects of cell wall modifications during fruit ripening. Crit Rev Food Sci, 39: 149–164.

Brummel, D.A., Harpster, M.H., 2001. Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants. Plant Mol Biol, 47: 311–340.

Callahan, A.M., Morgens, P.H., Wright, P., Nichols Jr., K.E., 1992. Comparison of pch313 (pTOM13 homolog) RNA accumulation during fruit softening and wounding of two phenotypically different peach cultivars. Plant Physiol, 100: 482–488.

Fishman, M.L., Levaj, B., Gillespie, D., 1993. Changes in the physiochemical properties of peach fruit pectin during on-tree ripening and storage. J Am Soc Hortic Sci, 118: 343–349.

Gao, H.Y., Chen, H.J., Chen, W.X., Song, L.L., Mao, J.L., Zhou, Y.J., Zhen, Y.H., 2009. Effects of harvest maturity on quality and chilling injury of juicy peaches during low temperature storage. Sci Agric Sin, 42: 612–618. (in Chinese)

Guo, L., Cai, Z.X., Zhang, B.B., Xu, J.L., Song, H.F., Ma, R.J., 2013. Fruit coloration and expression of some related genes in peach with external ABA treatment. Acta Bot Bor-Occid Sin, 33: 1750–1755. (in Chinese)

Guo, Y.P., Ma, H.L., Wang, G.X., Liang, L.S., 2009. Effect of the ethylene on the peach fruit quality and relative physiological mechanism during the MA storage and shelf life. J Northwest A&F Univ, 37: 34–37. (in Chinese)

Haji, T., Yaegaki, H., Yamaguchi, M., 2001. Changes in ethylene production and flesh firmness of melting, nonmelting and stonyhard peaches after harvest. J Jpn Soc Hortic Sci, 70: 458–459.

Haji, T., Yaegaki, H., Yamaguchi, M., 2003. Softening of stony hard peach by ethylene and the induction of endogenous ethylene by 1-aminocyclopropane-1-carboxylic acid (ACC). J Jpn Soc Hortic Sci, 72: 212–217.

Haji, T., Yaegaki, H., Yamaguchi, M., 2004. Varietal differences in the relationship between maturation characteristics, storage life and ethylene production in peach fruit. J Jpn Soc Hortic Sci, 73: 97–104.

Haji, T., Yaegaki, H., Yamaguchi, M., 2005. Inheritance and expression of fruit texture melting, non-melting and stony hard in peach. Sci Hortic, 105: 241–248.

Hayama, H., Shimada, T., Fujii, H., Ito, A., Kashimura, Y., 2006. Ethylene regulation of fruit softening and softening-related genes in peach. J Exp Bot, 57: 4071–4077.

Kan, J., Liu, J., Jin, C.H., 2012. Study on the relationship between peach fruit softening, cell wall degradation related glycosidase and ethylene biosynthesis. Sci Agric Sin, 45: 2931–2938. (in Chinese)

Kende, H., 1993. Ethylene biosynthesis. Annu Rev Plant Physiol, 44: 283–307.

Lester, D.R., Speirs, J., Orr, G., Brady, C.J., 1994. Peach (*Prunus persica*) endopolygalacturonase cDNA isolation and mRNA analysis in melting and nonmelting peach cultivars. Plant Physiol, 105: 225–231.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods, 25: 402–408.

Ma, S.S., Tang, Y., Wu, Ch.L., Liu, Y.L., Du, G.Y., 2003. Effect of 1-MCP and storage temperatures on respiration, ethylene production and fruit quality of peach and nectarine. Acta Hort Sin, 30: 525–529.

Mao, L.Ch., Zhang, Sh.L., 1999. Responses of polyamines and ethylene in peaches to chilling stress. Acta Hort Sin, 26: 360–363.

Mathooko, F.M., Tsunashima, Y., Owino, W.Z.O., Kubo, Y., Inaba, A., 2001. Regulation of genes encoding ethylene biosynthetic enzymes in peach (*Prunus persica* L.) fruit by carbon dioxide and 1-methylcyclopropene. Postharvest Biol Technol, 21: 265–281.

Shi, B.Sh., Zhuo, L.H., 2006. Modification and comparation of total RNA extraction methods from *Prunus cerasifera* leaves. Mol Plant Breeding, 4: 721–725. (in Chinese)

Tatsuki, M., Hiji, T., Yamaguchi, M., 2006. The involvement of 1-aminocyclopropane-1-carboxylic acid synthase isogene, *PpACS1*, in peach fruit softening. J Exp Bot, 57: 1281–1289.

Tong, Z.G., Gao, Z.H., Wang, F., Zhou, J., Zhang, Z., 2009. Selection of reliable reference genes for gene expression studies in peach using real-time PCR. BMC Mol Biol, 10: 71.

Tonutti, P., Bonghi, C., Ruperti, B., Tornielli, G.B., Ramina, A., 1997. Ethylene evolution and 1-aminocyclopropane-1-carboxylate oxidase gene expression during early development and ripening of peach fruit. J Am Soc Hortic Sci, 122: 642–647.

Wang, L.R., Zhu, G.R., Fang, W.Ch., Ma, R.J., Yu, M.L., Jiang, Q., Zhao, J.B., Guo, J.Y., Shen, Zh.J., 2005. Descriptors and data standard for peach. China Agriculture Press, Beijing, pp. 47.

Xu, Ch.J., Chen, K.S., Zhang, Sh.L., 1998. Advances in ethylene biosynthesis and its control. Chin Bull Bot, 15(S1): 54–61. (in Chinese)

Yang, S.F., Hoffman, N.E., 1984. Ethylene biosynthesis and its regulation in higher plants. Annu Rev Plant Physiol, 35: 155–189.

Yin, X.R., Zhang, B., Li, X., Chen, K.S., 2009. Ethylene signal transduction during fruit ripening and senescence. Acta Horticulturae Sinica, 36: 133–140.

Yoshida, M., 1976. Genetical studies on the fruit quality of peach varieties. III. Texture and keeping quality. Bull Fruit Tree Res Station, 3: 1–16.

Zarembinski, T.I., Theologis, A., 1994. Ethylene biosynthesis and action: A case of conservation. Plant Mol Biol, 26: 1579–1597.